Dormancy in peach (*Prunus persica*) flower buds. VI. Effects of gibberellins and an acylcyclohexanedione (trinexapac-ethyl) on bud morphogenesis in field experiments with orchard trees and on cuttings

Herminda Reinoso, Virginia Luna, Carlos Dauría, Richard P. Pharis, and Rubén Bottini

Abstract: The effects of several gibberellins (GAs), exo-16,17-dihydro GA_5, 2,2-dimethyl GA_4, and GA_3, and trinexapac-ethyl (an acylcyclohexanedione inhibitor of late-stage GA biosynthesis), were assessed for their effects on flower bud development during and after winter dormancy in peach (*Prunus persica* (L.) Batsch.) in three field trials and one experiment using cuttings. At late developmental stages, GA_3 hastened floral bud development and shortened the time to anthesis, whereas early-stage applications of GA_3 either had no effect or delayed floral bud development. In contrast, an exceptionally growth-active GA, 2,2-dimethyl GA_4, promoted floral bud development (tested only on cuttings) across a range of application dates. However, it also induced a high percentage of bud abscission and remaining buds had a necrotic gynoecium and alterations in the androecium. Surprisingly, trinexapac-ethyl also promoted floral bud development, although it was not as effective as GA_1. Trinexapac-ethyl-treated buds also showed morphological alterations and gynoecium necrosis. However, the best and most consistent treatment for enhancing floral bud development and hastening flower anthesis was 16,17-dihydro GA_5. It stimulated floral bud development in up to 80% of the treated buds. Further, the promotive effect of 16,17-dihydro GA_5 was maintained through to anthesis across three years of field experiments on intact trees, as well as with cuttings. Whether 16,17-dihydro GA_5, a competitive inhibitor of the 3β-hydroxylation step in GA biosynthesis, acts per se, acts via a metabolite (such as 16,17-dihydro GA_3), or acts by modifying endogenous GA metabolism is not yet known.

Key words: gibberellins, trinexapac-ethyl, floral bud morphogenesis, peach.

Résumé : Les auteurs ont évalué les effets de plusieurs gibbérellines (GAs), soit l'exo-16,17-dihydro GA_5, la 2,2-diméthyl GA_4, et la GA_3 ainsi que le trinexapac-éthyl (un inhibiteur de l'acylcyclohexanedione vers la fin de la biosynthèse des AG, sur le développement du bourgeon floral; ces essais ont été effectué au cours et après la dormance hivernale chez la pêche (*Prunus persica* (L.) Batsch.), dans le cadre de trois expériences conduites sur le terrain, et une expérience effectuée sur des boutures. Aux dernières étapes du développement, la GA_3 accélère le développement du bourgeon floral et raccourci la durée de l'anthèse, alors que des applications de GA_3 aux premiers stades restent sans effet, ou retardent le développement du bourgeon floral. Au contraire, une GA particulièrement active sur la croissance, la 2,2-diméthyl GA_4, stimule le développement du bourgeon floral (vérifié uniquement sur boutures) pour un ensemble de dates d'application. Cependant, il induit un fort pourcentage d'abscission des bourgeons, et les bourgeons qui restent montrent des gynécées nécrosés et des altérations de l'androcée. Ce qui est surprenant, le trinexapac-éthyl stimule également le développement du bourgeon floral, bien qu'il ne soit pas aussi efficace que la GA_1. Les bourgeons traités avec le trinexapac-éthyl montrent également des altérations morphologiques et une nécrose du gynécée. Enfin, le traitement le meilleure et le plus fiable pour stimuler le développement du bourgeon floral et accélérer l'anthèse est la 16,17-dihydro GA_5. Elle stimule le développement du bourgeon floral chez 80% des bourgeons traités. De plus, l'effet promoteur de la 16,17-dihydro GA_5 s'est maintenu tout au long de l'anthèse, au cours des trois années

Received 16 May 2001. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on 19 June 2002.

H. Reinoso. Laboratorio de Morfología Vegetal, Departamento de Ciencias Naturales, Universidad Nacional de Río Cuarto, 5800 Río Cuarto, Argentina.
V. Luna[1] and R. Bottini. Laboratorio de Fisiología Vegetal, Departamento de Ciencias Naturales, Universidad Nacional de Río Cuarto, 5800 Río Cuarto, Argentina.
C. Dauría.[2] Cátedra de Producción Frutícola, Departamento de Producción Vegetal, Universidad Nacional de Río Cuarto, 5800 Río Cuarto, Argentina.
R.P. Pharis. Plant Physiology Research Group, Department of Biological Sciences, University of Calgary, Calgary, AB T2N 1N4, Canada.

[1]Corresponding author (e-mail: vluna@exa.unrc.edu.ar).
[2]Deceased.

 DOI: 10.1139/B02-051

d'expérimentation sur le terrain portant sur des arbres intacts, aussi bien que sur des boutures. On ne sait pas si la 16,17-dihydro GA_5, un inhibiteur compétitif de l'étape 3β-hydroxylation lors de la synthèse des GA, agit en soi, agit via un métabolite (tel que la 16,17-dihydro GA_3) ou encore agit en modifiant le métabolisme endogène des GA.

Mots clés : gibbérellines, trinexapac-éthyl, morphogénèse du bourgeon floral, pêche.

[Traduit par la Rédaction]

Introduction

Understanding the physiology of dormancy in floral buds of woody angiosperms, especially fruit trees, is a matter of significant economic importance. Nonetheless, it is a poorly understood process, despite an extensive literature going back to the 1930s (Boysen-Jensen 1936; Hemberg 1949; Howard 1951; Doorenbos 1953; Samish 1954; Nitsch 1957; Chandler 1960; Eagles and Wareing 1964; Perry 1971; Wareing and Saunders 1971; Fuchigami et al. 1977; Couvillon and Erez 1985; Saure 1985; Powell 1987; Martin 1991).

Plant hormones and inhibitory substances were implicated early on in bud dormancy (Boysen-Jensen 1935; Hemberg 1949; Hendershott and Walker 1959; Cornforth et al. 1965; Okhuma et al. 1965). Once it was discovered that GA_3 applications could break dormancy under short days (Lockhart and Bonner 1957), the concept that dormancy is maintained by a balance between growth promoters (gibberellins (GAs)) and inhibitors (numerous) was soon put forward (Phillips 1962; Smith and Kefford 1964). Since this early work, many studies have shown that applied GAs (usually GA_3) can often, but not always, break floral bud dormancy in a wide range of woody angiosperms (Looney and Pharis 1985).

Endogenous levels of GAs and putative inhibitors have been examined for floral buds of several species (Correa et al. 1975; Bottini et al. 1976, 1978; Zimmermann et al. 1985). For peach (*Prunus persica* (L.) Batsch.), the endogenous GAs commonly classed as "growth effectors", GA_1 and GA_3, reached maximum levels when the flower buds were at their lowest rate of organ development (Luna et al. 1990, 1993; Luna 1993). Then, as anthesis approached, the levels of GA_1 and GA_3 decreased, while their likely precursor, GA_{20}, increased (Bottini and Luna 1993; Luna et al. 1993). To state it simplistically, endogenous concentrations of growth-active GAs within the peach floral bud are not directly linked to the rapid floral organ growth and differentiation that occurs just before anthesis. This of course ignores "balances" between GAs and inhibitory substances and also ignores the possibility of a more rapid turnover (catabolism) of growth-active GAs in the later stages of bud dormancy well before anthesis.

Nonetheless, the literature unequivocally shows that application of GAs, especially during the later stages of floral bud dormancy, can promote bud break in a range of woody angiosperm species (Walker and Donoho 1959; Hatch and Walker 1969; Couvillon and Hendershott 1974; Walser et al. 1981). However, GA_3 cannot entirely replace the chilling requirement of certain species, such as apple (Paiva and Robitaille 1978). Finally, when applied during earlier stages of peach flower bud dormancy, GA_3 can actually inhibit subsequent bud break (Luna et al. 1991; Basconsuelo et al. 1995).

Being able to control floral bud dormancy is important both for maintaining dormancy to prevent frost damage and for shortening it to allow for the growing of fruit trees, like apricot and peach, in warm climates. Because of this, we began an extensive examination of the effects of GA_3 dose and the timing of its application on floral bud dormancy. We also examined two other GAs of unique structure, i.e., 2,2-dimethyl GA_4, an exceptionally growth-active GA (Hoad et al. 1982), and the exo-isomer of 16,17-dihydro GA_5 (dihydro GA_5). Dihydro GA_5 has been found to have a unique ability to retard shoot growth in grasses (Evans et al. 1994*b*; Mander et al. 1998). However, for dicots, it often causes significant growth promotion (Brian et al. 1967). The growth inhibition by dihydro GA_5 occurs because the molecule acts as a competitive substrate inhibitor in GA biosynthesis (Takagi et al. 1994; Foster et al. 1997; Zhou and Pharis 1998). That is, dihydro GA_5 inhibits 3β-hydroxylation of GA_{20} to GA_1. Just how dihydro GA_5 promotes growth in dicots, however, is not well understood (see Discussion for possible mechanisms). Based on the growth-promotive effect of dihydro GA_5 in dicots (see above), we speculated that it might also promote floral organ expansion (e.g., bud break or anthesis).

Additionally, because of the unusual "retarding" effects of GA_3 on early developmental growth of peach bud floral organs (Basconsuelo et al. 1995), we also examined the effects of trinexapac-ethyl, an acylcyclohexanedione inhibitor of late-stage GA biosynthesis (Adams et al. 1991; Rademacher et al. 1992). That is, we postulated that reducing endogenous GA levels within the floral bud very early on with trinexapac-ethyl might hasten subsequent bud development.

Materials and methods

Field experiment 1: 1992

All field experiments utilized peach trees grown in an orchard at Río Cuarto, Argentina. Geographically, the location is 33°07′S, 64°14′W at 421 m altitude with a temperate thermal regime, i.e., average temperature for the coldest month (July) is 9.1°C and for the hottest month (January) is 23°C. The average minimal temperature in July is 3°C and the average maximal temperature for January is 29°C. Average day length is 10.9, 11.2, and 11.8 h in June, July, and August, respectively, with a total winter average of full sunlight being 53% of the daylight hours. Based on average rainfall, the area is classed as dry–subhumid with an average annual precipitation of 801.2 mm, rains being more abundant and frequent during the warm season (monsoonic regime).

For the first experiment, eight 10-year-old cv. Pen-Too peach trees were chosen. Each plant was divided into two different zones, each zone being considered as a block.

Applications of growth regulators were made to three sets of plants as follows. Set 1 (three plants, six plots) was treated initially on 22 May, with a repeat treatment on 27 May. Set 2 (three plants, six plots) was treated on 12 and 23 June. Set 3 (two plants, four plots) was treated on 3 and 10 July. The plant growth regulators were applied as follows. One-year-old limbs were selected from each plot for treatment and floral buds were "brush-painted" with approximately 10 μL of 95% ethanolic solutions of (*i*) GA_3 (90% purity) (Sigma Chemical Co., St. Louis, Mo.) at 5, 17, 55, 165, and 550 ppm, (*ii*) the exo-isomer of dihydro GA_5 at 5, 17, 55, 165, and 550 ppm, (*iii*) a 95% ethanol only control, and (*iv*) an untreated "true" control. The dihydro GA_5 was kindly supplied by Prof. L.N. Mander, Research School of Chemistry, Australian National University, Canberra, Australia. At the time each treatment was applied, separate buds were subsampled from the true controls in order to assess their stage of anatomical development. Floral buds of treatments *i–iv* were collected on the following dates: set 1, 1 and 12 June, 10 July, 3 and 14 August; set 2, 10 July, 3 and 14 August; set 3, 3 and 14 August. On each collection date, 12–15 buds were taken from each treatment and plot and fixed in FAA (formaldehyde – acetic acid – ethanol – water (10:5:50:35, v/v/v/v). The scales were then removed and buds assessed by histological examination according to Luna et al. (1990) and Reinoso et al. (2002). After flower bud swelling became evident in a majority of control buds (i.e., on 24 August and 1 September), only phenological observations were made. Phenological stages were determined according to the basic nomenclature of Baggliolini (1952) as described in Reinoso et al. (2002).

Field experiment 2: 1995

The second field experiment was carried out with nine 13-year-old peach trees (cv. Pen-Too) on different plants from the same orchard at Río Cuarto, Argentina. Each plant was divided into three separate zones that were visually similar, each zone being considered as a block. Applications of growth regulators were made to three sets of plants (three plants, nine plots for each set). Set 1 was treated initially on 10 May, with a repeat treatment on 17 May. Set 2 was treated on 9 and 14 June. Set 3 was treated on 7 and 17 July. As in field experiment 1, 1-year-old limbs were selected from each plot for treatment. Floral buds were brush-painted with approximately 10 μL of 95% ethanolic solutions of (*i*) GA_3 at 55 and 165 ppm, (*ii*) dihydro GA_5 at 55 and 165 ppm, (*iii*) a 95% ethanol only control, and (*iv*) an untreated true control. Collections for floral bud assessment and the protocol used were as above for field experiment 1, except that harvests were made as follows: set 1, 14 June, 4 July, 8 and 23 August; set 2, 4 July, 8 and 23 August; set 3, 8 and 14 August. After flower bud swelling became evident on most control buds (i.e., on 1 and 8 September), only phenological observations were made according to the criteria listed for field experiment 1.

Field experiment 3: 1996

The third field experiment was carried out with three 14-year-old peach trees (cv. Pen-Too). These were different plants from those used in field experiments 1 and 2 but were in the same orchard. Each plant was divided into three different zones (one plant, three plots). The protocol used was similar to that of field experiment 2, with the following exceptions: the GA applications were made on 5 July, with a repeat treatment on 17 July. Harvests for anatomical assessments were made on 9 August and phenological observations were made on 24 August according to the criteria listed for field experiment 1.

Statistical analysis

Quantitative data from our assessments of flowering stage were analyzed using STATA™ version 5.0 as a factorial assay, considering each plant as a block with more than one replication. The analyzed variable was percentage of open flowers. ANOVA was used to determine the significance of differences between control and treated plants for each application date. Flowering data were arcsine transformed before statistical analysis to ensure homogeneity of variance. Normality was verified with the Shapiro–Wilk test (p = 0.193, 0.170, and 0.301 for field experiments 1, 2, and 3 respectively). Homogeneity of variance was verified with the Bartlett test. For field experiments 1 and 2, Dunnet and Tukey tests were used to determine significance between control and treated plants and between treatments, respectively. For field experiment 3, the Scheffé test was applied because of data frequency disparity.

Experiment with detached branches: 1994

One-year-old limbs were detached from trees in the same orchard utilized for field experiment 1. These trees had never been treated. The detached limbs were then cut into 10- to 12-cm sections (cuttings), each cutting having one floral bud present at the second node (proximal to the detached apical end).

After sealing the upper cut with pure lanolin, 20–22 cuttings per treatment were planted in pots containing sand–vermiculite (1:1) as described by Basconsuelo et al. (1995) and then placed under continuous fluorescent light (220 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 20–25°C and 100% relative humidity. Plant growth regulators were applied in ethanol, as above, on the day of planting (i.e., on 6 and 21 June, 8 and 25 July). They consisted of GA_3 (1 ppm), dihydro GA_5 (1 ppm), 2,2-dimethyl GA_4 (1 ppm), and trinexapac-ethyl (50 ppm). The 2,2-dimethyl GA_4 was a gift from Prof. L.N. Mander (Research School of Chemistry, Australian National University, Canberra, Australia) and the trinexapac-ethyl was provided by Ciba-Geigy (Dorval, Que.). Applications were made by microsyringe in order to apply 3 μL of the above solutions to each bud every 3 days over a 15-day period. At the time each treatment was made, separate buds were subsampled from the true control trees in order to assess their stage of anatomical development. Histological assessments were always made 18 days after application of the plant growth regulator. As for field experiments 1–3, there were controls treated with ethanol only and untreated true controls. Because no treatment reached flowering, statistical analysis was not performed.

Results

Field experiment 1

Assessment of anatomical development and phenological stages is shown for early (Table 1), middle (Table 2), and late (Table 3) application dates for three doses (55, 165, and

Table 1. Field experiment 1, 1992, showing anatomical (1–10) and phenological (B–F) predominant stages observed during development of peach floral buds treated with GA_3 and dihydro GA_5 at three doses on each of 22 and 27 May (at stage 2).

	Days after first treatment					
	Anatomical stages				Phenological stages	
Gibberellins (ppm)	21	49	73	84	105	113
GA_3						
0	3	4	6	8	D	F *a*
55	5	6	8	10(b)	D	F *b*
165	5	6* AB	6 AB	8 AB	AB-D	AB-D_2
550	4*	AB	AB	AB	AB	AB
Dihydro GA_5						
55	4	5	7	10(a)	D_2	F *abc*
165	4	6	8	10(b)	D_2	F *c*
550	4	5	6	9	D_2	F *abc*

Note: See Reinoso et al. (2002) for a description of the stages. AB, bud abscission after pedicel elongation. Each value is the average anatomical stage seen within a sample of 12–15 buds. Phenological stage F assessments followed by the same letter do not differ significantly at $p > 0.05$ (Dunnet and Tukey tests).
*Necrotic bud.

550 ppm) of GA_3 and dihydro GA_5. Both GAs were also tested at two lower doses (5 and 17 ppm). However, effects of those doses did not differ from control values and thus are not presented. Ethanol controls and untreated controls had similar assessment scores; hence, the controls shown represent untreated trees.

Treatments with GA_3 at the early and middle application dates (22 and 27 May or 12 and 23 June) caused considerable necrosis in all whorls at the two higher doses (165 and 550 ppm) (Fig. 2), with a high rate of bud abscission occurring after pedicel elongation (Tables 1 and 2). Thus, the excessive growth that GA_3 induced in the floral pedicels appeared to have effectively "pushed" the bud away from the protective scales, and this lack of protection caused their abscission (Fig. 1). Nevertheless, at the middle application date, a dose of 165 ppm caused less abscission, and flowering porcentage was similar to controls. A lower dose of GA_3 (55 ppm) at both the early and middle application dates, however, tended to promote anatomical (Tables 1 and 2) but not phenological development. Instead, at the early application date, flowering was delayed (Table 1).

The late application dates (3 and 10 July) for GA_3 tended to promote anatomical development, especially at the day 42 assessment. For the phenological stage, only the highest GA_3 doses showed an effect (Table 3) and this reflects only a promotion of final pedicel elongation.

Early (22 and 27 May) (Table 1) and middle (12 and 23 June) (Table 2) applications of dihydro GA_5 considerably accelerated differentiation of floral buds at all doses but especially at the two lower doses of 55 and 165 ppm (Tables 1 and 2). This promotive effect persisted throughout the assessment period, rendering it an anticipated flowering (statistically significant) in the case of buds treated with 165 ppm (Tables 1 and 2). However, buds treated on 3 and 10 July with dihydro GA_5 did not differ from controls (Table 3).

Table 2. Field experiment 1, 1992, showing anatomical (1–10) and phenological (B–F) predominant stages observed during development of peach floral buds treated with GA_3 and dihydro GA_5 at three doses on each of 12 and 23 June (at stage 3).

	Days after first treatment				
	Anatomical stages			Phenological stages	
Gibberellins (ppm)	28	52	64	85	93
GA_3					
0	4	5	8	D	F *a*
55	4	4	10(a)	D_1	F *ab*
165	5	7 AB	10(b)	D_2	F *a*
550	6*	7* AB	AB	AB	AB
Dihydro GA_5					
55	4	6	10(a)	D_2	F *ab*
165	4	6	10(a)		F *b*
550	4	6	9	D_2	F *ab*

Note: See Reinoso et al. (2002) for a description of the stages. AB, bud abscission after pedicel elongation. Each value is the average anatomical stage seen within a sample of 12–15 buds. Phenological stage F assessments followed the same letter do not differ significantly at $p > 0.05$ (Dunnet and Tukey tests).
*Necrotic bud.

Table 3. Field experiment 1, 1992, showing anatomical (1–10) and phenological (B–F) predominant stages observed during development of peach floral buds treated with GA_3 and dihydro GA_5 at three doses on each of 3 and 10 July (at stage 3).

	Days after first treatment			
	Anatomical stages		Phenological stages	
Gibberellins (ppm)	31	42	63	81
GA_3				
0	6	7	D	F *a*
55	6	8	D	F *a*
165	6	8*	D	F *a*
550	6	8*	D	F *a*
Dihydro GA_5				
55	5	8	D	F *a*
165	5	8	D	F *a*
550	4	5	D	F *a*

Note: See Reinoso et al. (2002) for a description of the stages. Each value is the average anatomical stage seen within a sample of 12–15 buds. Phenological stage F assessments followed by the same letter do not differ significantly at $p > 0.05$ (Dunnet test).
*Bud with elongated pedicel.

Field experiment 2

For GA_3 applied at the earliest dates (10 and 17 May), anatomical stages were slightly more advanced at assessment days 35 and 55, but this treatment had retarded floral bud development when assessed at days 90 and 105. Nevertheless, at assessment day 121, flowering had no difference

Figs. 1–3. Peach flower buds treated with GA_3 at different doses. Fig. 1. Dose of 550 ppm applied on 22 and 27 May, Field experiment 1. Unprotected flower bud (b) due to pedicel (p) elongation 21 days after treatment. Scale bar = 10 mm. Fig. 2. Dose of 550 ppm applied on 12 and 23 June, Field experiment 1. Photomicrograph of a longitudinal section of a flower bud with necrosis symptoms (dark areas) in its whorls 28 days after treatment. Scale bar = 600 μm. Fig. 3. Dose of 165 ppm applied on 9 and 14 June, Field experiment 2. Pollen grains of different size from buds collected 74 days after treatment. Scale bar = 20 μm.

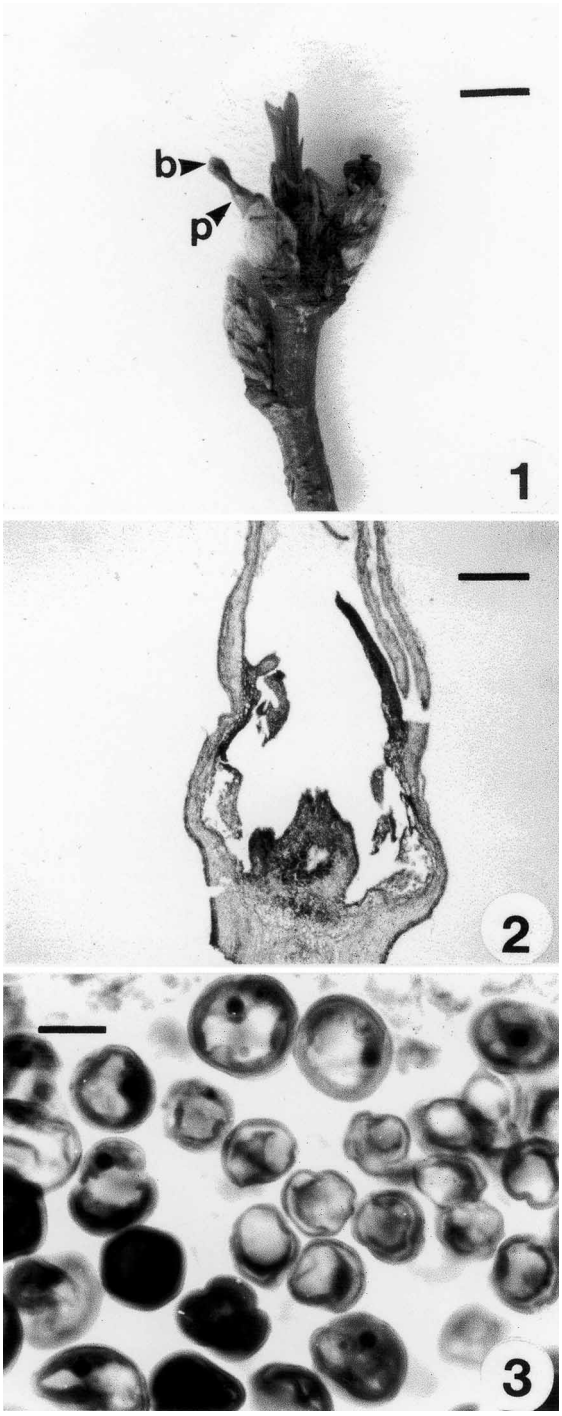


Table 4. Field experiment 2, 1995, showing anatomical (1–10) and phenological (B–F) predominant stages observed during development of peach floral buds treated with GA_3 and dihydro GA_5 at two doses on each of 10 and 17 May (at stage 1).

	Days after first treatment					
	Anatomical stages				Phenological stages	
Gibberellins (ppm)	35	55	90	105	114	121
GA_3						
0	2	3*	6	8	C	F *a*
55	3*	4	5	6	B	F *ab*
165	3*	4	5	7	C	F *ab*
Dihydro GA_5						
55	2	3	6	8	C	F *ab*
165	3*	3	6	10(a)	D_1	F *b*

Note: See Reinoso et al. (2002) for a description of the stages. Each value is the average anatomical stage seen within a sample of 12–15 buds. Phenological stage F assessments followed by the same letter do not differ significantly at $p > 0.05$ (Dunnet and Tukey tests).
*Bud without seminal rudiment.

form controls (Table 4). In comparison, dihydro GA_5 also tended to promote early-stage floral bud development (assessment at days 35 and 55) but had no negative effect on late-stage development at day 90 (Table 4). In fact, dihydro GA_5 appreciably promoted late-stage floral bud development, especially for the 165-ppm dose (day 105 assessment, Table 4), which was the only treatment that caused a statistically significant increase in flowering.

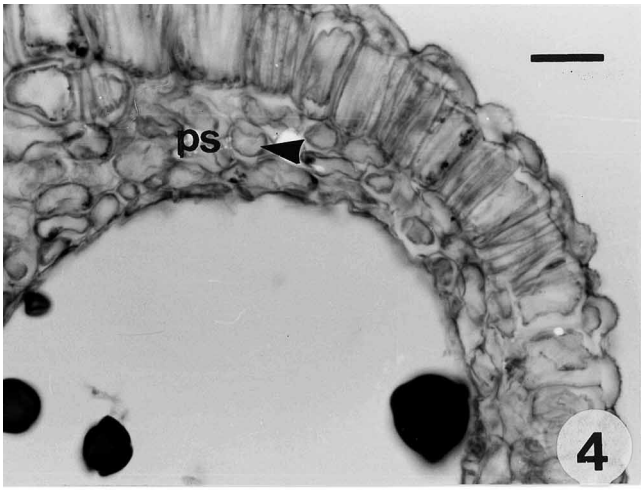
Interestingly, for dihydro-GA_5-treated trees, anatomical development of the endothecium cells and pollen grain characteristics correspond to stage 10 buds in spite of the fact that the middle layers are not yet compressed (Fig. 4).

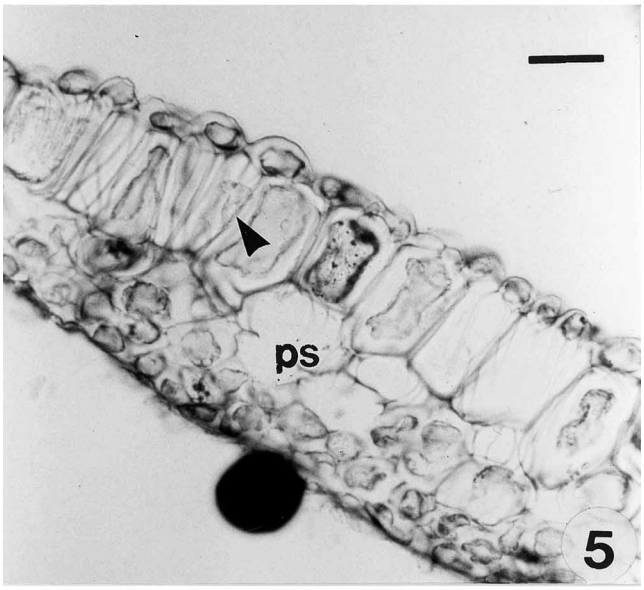
For the middle treatment dates, 9 and 14 June, GA_3 application appreciably enhanced bud development at assessment day 59 (165 ppm), but there was a wide range for this enhancement response (anatomical stages 5–9 could be seen, Table 5).

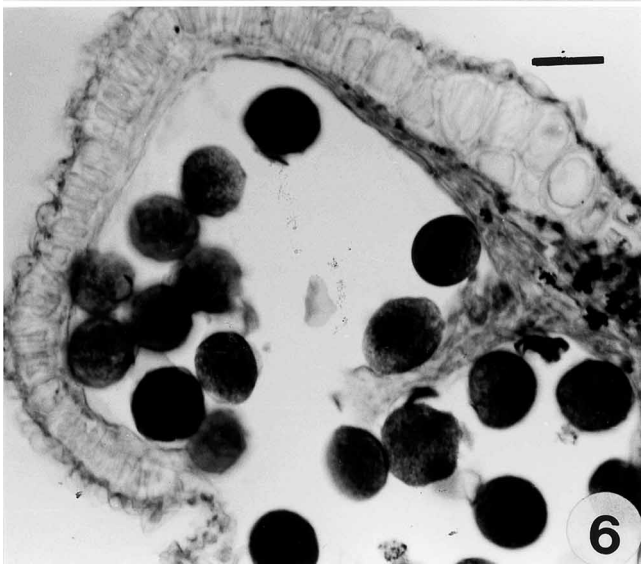
By assessment day 74, both low and high doses of GA_3 caused marked enhancement of floral bud development relative to the control (Table 5). In comparison, dihydro GA_5 caused only a slight enhancement of floral bud development in some buds at the low dose (55 ppm). However, the high dose of dihydro GA_5 broadened this enhancement to all buds (anatomical stage 6, Table 5). Thus, by assessment day 74, the high doses of dihydro GA_5 had caused marked floral bud development. This dihydro GA_5 effect was comparable with that obtained with GA_3 when differences in control floral bud development were taken into account (Table 5).

At 55 and 165 ppm GA_3, a noticeable heterogeneity in pollen grain size was observed (Fig. 3). However, subsequent phenological development was affected very little by GA_3 or dihydro GA_5 at each of assessment days 83 and 90 (Table 5). Thus, in this year, late-stage development occurred quite rapidly for controls and they caught up to the dihydro GA_5 treated buds between assessment days 74 and 90.

Finally, for the latest dates of hormone application, 7 and 17 July, GA_3 caused a pronounced promotion of floral bud development at each of the low and high doses on both of







Figs. 4–6. Peach flower buds treated with dihydro GA_5 at different doses. Fig. 4. Dose of 165 ppm applied on 10 and 17 May, Field experiment 2. Transverse section of a mature microsporangium wall collected 105 days after treatment when parietal strata (ps) are still intact. Scale bar = 30 μm. Fig. 5. Dose of 165 ppm applied on 5 and 12 July, Field experiment 3. Transverse section of a microsporangium wall (stage 9); endothecium cells were large but the fibrous thickening of their walls was very weak (arrowhead) or absent and the parietal strata were hypertrophic (Fig. 5). Buds were collected 35 days after treatment. Scale bar = 30 μm. Fig. 6. Dose of 165 ppm applied on 5 and 12 July, Field experiment 3. Transverse section of a dehiscent anther where the parietal strata have disappeared and normal microspores are present. Scale bar = 50 μm.

Table 5. Field experiment 2, 1995, showing anatomical (1–10) and phenological (B–F) predominant stages observed during development of peach floral buds treated with GA_3 and dihydro GA_5 at two doses on each of 9 and 14 June (at stages 2 and 3).

	Days after first treatment				
	Anatomical stages			Phenological stages	
Gibberellins (ppm)	24	59	74	83	90
GA_3					
0	3	5	8	C	F *a*
55	4	7	9	D	F *a*
165	4	8	10(a)	D	F *a*
Dihydro GA_5					
55	3	6	8	D	F *a*
165	3	6	10(a)	D	F *a*

Note: See Reinoso et al. (2002) for a description of the stages. Each value is the average anatomical stage seen within a sample of 12–15 buds. Phenological stage F assessments followed by the same letter do not differ significantly at $p > 0.05$ (Dunnet test).

assessment days 32 and 47 (Table 6). On day 32, the buds treated with lower doses of GA_3 had reached stage 7. A slightly less pronounced promotion of floral bud development was also seen for the late application of dihydro GA_5, most buds being in stage 6 (Table 4).

The exceptional promotion by GA_3 was also seen for flower development at both doses on assessment days 56 and 63 (Table 6), with a similar but slightly less pronounced promotion being seen for 16,17-dihydro GA_5 (Table 6). Both GAs, however, exhibited highly significant promoting effects on early floral bud development and on flower development (Tables 4 and 7).

Field experiment 3

As for the 1995 trial, GA_3 showed an appreciable promotion of floral bud development at assessment day 35 (where differences in microspore size were also observed). Phenological development (which occurred very early in 1996) was also very appreciably promoted by GA_3 applications (i.e., assessment day 49, Table 8). In contrast, late-stage application of dihydro GA_5 gave a somewhat lesser promotive effect of floral bud development, relative to GA_3,

Table 6. Field experiment 2, 1995, showing anatomical (1–10) and phenological (B–F) predominant stages observed during development of peach floral buds treated with GA_3 and dihydro GA_5 at two doses on each of 7 and 17 July (at stages 3–5).

	Days after first treatment			
	Anatomical stages		Phenological stages	
Gibberellins (ppm)	32	47	56	63
GA_3				
0	5	7	C	F *a*
55	7	9	D_2	F *b*
165	7	10(a)	D_2	F *b*
Dihydro GA_5				
55	6	9	D	F *b*
165	6	9	D	F *b*

Note: See Reinoso et al. (2002) for a description of the stages. Each value is the average anatomical stage seen within a sample of 12–15 buds. Phenological stage F assessments followed by the same letter do not differ significantly at $p > 0.05$ (Dunnet and Tukey tests).

at assessment day 35 (Table 8). Here, at 165 ppm dihydro GA_5, endothecium cells were large, but the fibrous thickening of their walls was very weak or absent and the parietal strata were hypertrophic (Fig. 5). However, the enhancement of phenotype development by dihydro GA_5 was similar to that induced by GA_3 (low dose) and only slightly less than that induced by the high dose of GA_3 (Table 8). Indeed, flowers collected at assessment day 49 showed dehiscent anthers and apparently normal microspores (Fig. 6).

Hence, for these two years (1995 and 1996), both GA_3 and dihydro GA_5, when applied at the late date, yielded an appreciably enhanced anatomical and phenological development of the flower buds and flowers, respectively. As controls flowered a week later than treated buds, statistical differences were established between treatments, resulting in 165 ppm GA_3 being significantly more promoting than the others.

Experiments using cuttings

The experiment in 1994 used cuttings. Here, treatment with GA_3, dihydro GA_5, 2,2-dimethyl GA_4, or trinexapac-ethyl was accomplished under laboratory conditions. The protocol was similar to that of the field experiments, with cuttings being taken and immediately treated early (6 June), middle (21 June), late (8 July), or very late (25 July). In all cases, control floral bud development generally proceeded only to stages 4–6 (Table 7). Appreciable differences in the rapidity of floral bud development, however, could be obtained by modifying date of cutting and GA application (Table 7).

Thus, for the early treatment, GA_3 retarded floral bud and flower development but gave a moderate promotive effect at the last date (Table 7). Dihydro GA_5, however, caused exceptional promotion of floral bud development at the two earliest dates (6 or 21 June) and was equal to GA_3 on 8 July but was less effective than GA_3 at the latest date (Table 7).

2,2-Dimethyl GA_4 also promoted floral bud development when applied at the earliest date but caused appreciable abscission when applied on 21 June or 8 July (Table 7). Late applications of 2,2-dimethyl GA_4 also increased the frequency of buds with anomalies in the androecium (i.e., empty or collapsed microspores, Table 7).

Trinexapac-ethyl had no effect on floral bud development at the earliest application date and was slightly better than GA_3 at the middle application date but was not quite as good as GA_3 at the two later application dates (Table 7). For the earlier dates, trinexapac-ethyl was not nearly as effective as dihydro GA_5 (Table 7). For the last application date, despite the fact that anatomical and phenological development of the flower was highly advanced relative to controls (Table 7), the fertile whorls in trinexapac-ethyl-treated buds showed severe androecium alterations and gynoecium necrosis.

Discussion

In the present work, we have investigated the effects of applied GAs and a late-stage GA biosynthesis inhibitor on development of dormant peach floral buds through to anthesis, taking into account the anatomical and phenological stages as described in Reinoso et al. (2002). Our earlier work (Basconsuelo et al. 1995), using young peach floral buds (early autumn, March, April in the Southern Hemisphere), on cuttings held at room temperature showed that floral bud development could be delayed by early applications of GA_3. In contrast, if GA_3 application was delayed, the opposite effect was seen, with development and maturation being hastened (Basconsuelo et al. 1995). In the current study, we have confirmed the delaying effect of early applications of GA_3 and again noted that late applications enhanced floral bud development (Table 7).

Since foliage remained on the peach trees into June, our GA applications in early June may have prolonged leaf retention, which, together with decreasing day length, could have contributed to an increase in inhibitors such as naringenin (Bottini et al. 1978; Luna et al. 1993). Thus, the delay seen in anatomical floral bud development (Table 4) from early May applications of GA_3 could be indirect. However, late June applications of GA_3 to cuttings hastened floral bud development (Table 7). Since May and early June are periods when naringenin levels in the buds are still decreasing but endogenous GA_3 levels are quite high (Luna et al. 1993), application of exogenous GA may also result in feedback inhibition of endogenous GA biosynthesis. This could subsequently slow or even prevent floral bud anthesis. For example, negative feedback regulation of GA C-20 oxidase was recently demonstrated in stems of *Arabidopsis* by application of bioactive GAs, such as GA_3 (Xu et al. 1999).

However, exogenous application of GA_3 in July hastened floral bud development, causing a rapid differentiation of pollen mother cells and their meiosis (stage 6). This occurred not only for cuttings (Table 7) but also for orchard trees in situ (Tables 3, 6, and 8). In July, endogenous GA_3 levels are low, as are levels of naringenin (Luna et al. 1993). Hence, the GA_3 applied in July could be supplementing very low endogenous levels. Thus, in the near absence of inhibitors such as naringenin, a much higher proportion of floral buds would be expected to develop through to anthesis.

Table 7. Experiment using cuttings, 1994, showing average anatomical stage reached (1–10) and percentage of cuttings with buds at these stages.

	6 June		18 days later	
	Stage	%	Stage	%
Control	2	70	3	66
	3	30	4–6	34
Ethanol			3	60
			6	40
GA_3 (1 ppm)			2	60
			3	40
Dihydro GA_5 (1 ppm)			3	28
			5–6	44
			8	28
2,2-Dimethyl GA_4 (1 ppm)			3	60
			6	25
			7	15
Trinexapac-ethyl (50 ppm)			3–4	70
			5–6	30
	21 June		18 days later	
	Stage	%	Stage	%
Control	3	100	4	80
			5	20
Ethanol			4	100
GA_3 (1 ppm)			4	25
			6	45
			Faded	30
Dihydro GA_5 (1 ppm)			6	30
			10(a)	20
			Faded	50
2,2-Dimethyl GA_4 (1 ppm)			AB	60
			4	40
Trinexapac-ethyl (50 ppm)			3	12
			6–7	38
			Faded	50
	8 July		18 days later	
	Stage	%	Stage	%
Control	2	20	3–4	80
	3	80	6	20
Ethanol			3	90
			6	10
GA_3 (1 ppm)			4	35
			6	25
			Faded	40
Dihydro GA_5 (1 ppm)			2–3	10
			4–5	30
			6–7	35
			Faded	25
2,2-Dimethyl GA_4 (1 ppm)			AB	20
			4-5	60
			8*	20
Trinexapac-ethyl (50 ppm)			3	45
			6	15
			Faded	40
	25 July		18 days later	
	Stage	%	Stage	%
Control	3	30	3	40
	4	70	5–6	30
			Faded	30
Ethanol			3	65
			5–6	35
GA_3 (1 ppm)			3–4	60
			10(a)	15
			Faded	25
Dihydro GA_5 (1 ppm)			4–5	50
			6–7	25
			Faded	25
2,2-Dimethyl GA_4 (1 ppm)			6	34
			8*	38
			Faded	28
Trinexapac-ethyl (50 ppm)			5–6	45
			8–9†	25
			10(a)†	30

Note: Cuttings were treated with GA_3, dihydro GA_5, 2,2 dimethyl GA_4, or trinexapac-ethyl at various dates and then observed over 18 days under laboratory conditions. See Reinoso et al. (2002) for a description of the stages. AB, bud abscission. Each value is the average stage or range of stages seen within a sample of 12–15 buds.

*Buds with severe anatomical alteration in androecium.

†Buds with necrotic gynoecium and alterations in androecium.

Detrimental effects of exogenous application of GA_3, especially high doses, include anomalous development, necrosis, and a delay in floral bud break (anthesis). Such effects were first noted many years ago (Donoho and Walker 1957) and were also apparent in the present study (Tables 1 and 2). In one of these early experiments on *Prunus avium* (Brian et al. 1959), trees that were on the periphery of the GA_3 spray pattern (and thus had received a somewhat lower dose of GA_3) showed a transient (3-week) delay in anthesis but otherwise produced apparently normal flowers. Thus, the literature on retarding effects of early-winter applications of GA_3 is confirmed across a number of decades. However, it was the promotive effects of applied GA_3 on floral bud development and anthesis that interested us. Thus, when two unique GA structures (2,2-dimethyl GA_4 and 16,17-dihydro GA_5) became available, we began a series of experiments comparing their effects with those of GA_3.

Table 7 summarizes the effects of 2,2-dimethyl GA_4 relative to GA_3 using cuttings. While toxicity occurred, this highly growth-active GA derivative (Hoad et al. 1982) obviously possessed a very good ability to promote floral bud development (but not anthesis), even when applied as early as 6 June and as late as 25 July (Table 7). Best promotion (38%) was in July, but toxicity was also severe then, with numerous malformations and necrosis, especially in the androecium. Thus, very few of the buds where floral bud development was promoted by 2,2-dimethyl GA_4 continued on to complete anthesis. It is worth noting that 2,2 dimethyl GA_4 has also been shown to be highly effective in promoting floral bud development in a GA-deficient mutant of *Arabidopsis* (Goto and Pharis 1999).

Table 8. Field experiment 3, 1996, showing anatomical (1–10) and phenological (B–F) predominant stages observed during development of peach floral buds treated with GA_3 and dihydro GA_5 at two doses on each of 5 and 12 July (at stages 2 and 3).

Gibberellins (ppm)	Anatomical stages, 35 days after first treatment	Phenological stages, 49 days after first treatment
GA_3		
0	6	D
55	8	F *a*
165	9	F *b*
Dihydro GA_5		
55	6	F *a*
165	8	F *a*

Note: See Reinoso et al. (2002) for a description of the stages. Each value is the average anatomical stage seen within a sample of 12–15 buds. Phenological stage F assessments followed by the same letter do not differ significantly at $p > 0.05$ (Scheffé test).

However, our most interesting results were obtained with the exo-isomer of 16,17-dihydro GA_5, both with isolated cuttings and for trees in situ. This ring D modified GA_5 derivative is not only florigenic in grasses (Evans et al. 1994*b*) but also in dicots (Evans et al. 1993), including trees (Ben-Tal et al. 1994; Clemens et al. 1994). Hence, one aspect of its florigenicity in trees may involve effects on either or both early and late floral bud differentiation.

A wide range of doses of dihydro GA_5 stimulated the development of peach floral buds, even when applied in May–June, and this promotive effect was maintained through to flower bud anthesis (Tables 1, 2, and 4). Interestingly, applied dihydro GA_5 promoted bud development only after meiosis of the pollen mother cells had occurred and vascular connections between the buds and stem were complete. This is in direct contrast with GA_3, which stimulates maturation of the pollen mother cells prior to meiosis.

Evidence to date with regard to the floral inductive effects of dihydro GA_5 in the grass *Lolium temulentum* strongly implies a per se activity on the potential floral apex (Evans et al. 1994*b*). Thus, its florigenicity is likely not gained through any effect as a competitive inhibitor of GA 3β-hydroxylases, as occurs in vegetative tissue of monocots (Evans et al. 1994*a*; Takagi et al. 1994; Foster et al. 1997; Zhou and Pharis 1998).

However, events taking place within the dormant peach bud are well past the floral induction stage. Ring D modified GA_5 derivatives not only can inhibit 3β-hydroxylation (as readily seen for monocots), but they may also influence GA catabolism (e.g., accumulation of 2β-hydroxylated GAs; Foster et al. 1997) and C-16 diols (D. Pearce, R. Pharis, M. Takagi, R. Zhou, unpublished manuscript). If this were the case, the net effect of applied dihydro GA_5, for this dicot at least, could be an increase in precursors (such as GA_{20}) to endogenous bioactive GAs. For example, blocking either or both of GA_{29} and GA_{20} C-16 diol (C-16,17-dihydrodihydroxy) formation would likely increase the level of endogenous GA_{20}. A surfeit in GA_{20} could effectively yield a net increase in endogenous growth-active GAs within the peach floral bud. That is, the primary effect of dihydro GA_5 as a competitive inhibitor of 3β-hydroxylation (which leads to reduced GA_1 levels in monocots) could be overwhelmed, allowing for increasd metabolic flow of GA_{20} to GA_1, the per se growth-active GA. The net result, then, for growing organs within the peach flower bud could be an increase in growth-active GAs such as GA_1 and GA_3. Alternatively, applied dihydro GA_5 could (speculatively) be 3β-hydroxylated to dihydro GA_3, the latter being a low-level growth promoter (Evans et al. 1994*a*; L. Janzen, R. Pharis, unpublished manuscript). Any or all of the above explanations would be consistent with the modest to large increases in growth of dicots that are seen after application of dihydro GA_5 (Brian et al. 1967; R. Pharis, J.B. Reid, R. Zhou, unpublished manuscript). Thus, the exceptional ability of dihydro GA_5 to mimic (or excel) applied GA_3 in peach floral bud development and anthesis could be due to a wide range of (currently speculative) mechanisms.

In summary, the response of the peach floral bud to applied GAs (and likely to changes in levels of its own endogenous GAs) is complex and depends very much on stage of anatomical development. Here, then, we might suspect an interaction between endogenous inhibitors, such as naringenin (Luna et al. 1993), and endogenous GA levels. With applied GA_3, we would also expect possible feedback inhibition of endogenous GA_3 biosynthesis to occur if too-high doses of GA_3 are used, i.e., as we saw in the May field experiments or in June with cuttings. Such a blocking effect on subsequent endogenous GA biosynthesis would effectively retard floral bud anthesis, even while promoting early bud development. However, for late applications of exogenous GA_3, when endogenous GA_3 levels are low (Luna et al. 1990, 1993; Bottini and Luna 1993), we would expect both bud development and anthesis to be promoted.

Acknowledgements

The study was supported with funds from CONICET (PID-88-205-1593 and PIP 4953) and from Secyt Universidad Nacional de Río Cuarto, Argentina, to R. Bottini and funds from the Natural Sciences and Engineering Research Council of Canada to R.P. Pharis. The helpful technical assistance of J. Bogino and O. Masciarelli is also gratefully acknowledged.

References

Adams, R., Kerber, E., Pfister, K., and Weiler, E. 1991. Studies on the action of a new retardant CGA 163935 (Cimectacarb). *In* Progress on plant growth regulation. *Edited by* C.M. Karssen, L.C. van Loon, and D. Vreugdenhil. Kluwer Academic Publishers, Dordrecht, Netherlands. pp. 818–827.

Baggliolini, M. 1952. Les stades repérés des arbres fruitiers à noyau. Rev. Romande Agric. Vitic. Arboric. **8**: 3–4.

Basconsuelo, S., Reinoso, H., Lorenzo, E., and Bottini, R. 1995. Dormancy in peach (*Prunus persica* L.) flower buds. IV. Floral bud morphogenesis as influenced by chilling and gibberellin A_3. Plant Growth Regul. **16**: 113–119.

Ben-Tal, Y., Erner, Y., Pharis, R.P., and Mander, L.N. 1994. Flower induction by artificial gibberellins. Twenty-fourth International

Horticultural Congress, Kyoto, Japan, August 1994. International Society for Horticultural Sciences, Leuven, Belgium. (Abstr.)

Bottini, R., and Luna, V. 1993. Dormancy in floral buds of deciduous fruit trees. *In* Current topics in plant physiology. Vol. 1. Council of Scientific Research Integration, Trivandrum, India. pp. 147–159.

Bottini, R., de Bottini, G.A., and Correa, N.S, 1976. Changes in the levels of growth inhibitors and gibberellin-like substances during dormancy of peach flower buds. Phyton, **34**: 157–167.

Bottini, R., Goleniowski, M., and Correa, N. 1978. Levels of growth inhibitors in leaves, cambial tissue and flower buds during dormancy in peach trees. Effect of exogenous gibberellic. Phyton, **36**: 17–21.

Boysen-Jensen, P. 1936. Growth hormones in plants. *Translated by* G.S. Avery and P.R. Burkholder. McGraw-Hill, New York.

Brian, P.W., Petty, J.H., and Richmond, P.T. 1959. Extended dormancy of deciduous woody plant treated in autumn with gibberellic acid. Nature (London), **184**: 69.

Brian, P.W., Grove, J.F., and Mulholland, T.P.C. 1967. Relationships between structure and growth promoting activity of the gibberellins and some allied compounds, in four test systems. Phytochemistry, **6**: 1475–1499.

Chandler, W.H. 1960. Some studies of rest in apple trees. Proc. Am. Soc. Hort. Sci. **76**: 1–10.

Clemens, J., Jameson, P.E., Bannister, P., and Pharis, R.P. 1995. Gibberellins and bud break, vegetative shoot growth and flowering in *Metrosideros collina* cv. Tahiti. Plant Growth Regul. **16**: 161–171.

Cornforth, J.W., Milborrow, B.V., Ryback, G., and Wareing, P.F. 1965. Identity of sycamore "dormin" with abscisin II. Nature (London), **205**: 1269–1270.

Correa, N.S., Bottini, R., Bottini, G.A., Goleniowski, M., and Gordon, L. 1975. Cambios en los niveles de inhibidores del crecimiento y giberelinas endógenas durante el reposo vegetativo de yemas de duraznero. Phyton, **33**: 193-204.

Couvillon, G.A., and Erez, A. 1985. Influence of prolonged exposure to chilling temperatures on bud break and heat requirement for bloom of several fruit species. J. Am. Soc. Hortic. Sci. **110**: 47–50.

Couvillon, G.A., and Hendershott, CH. 1974. A characterization of the "after-rest" period of flower buds of two peach cultivars of different chilling requirements. J. Am. Soc. Hortic. Sci. **99**: 23–26.

Donoho, C.W., and Walker, D.R. 1957. Effect of gibberellic acid on breaking dormancy of rest period in Elberta peach. Science (Washington, D.C.), **126**: 1178–1179.

Doorenbos, J. 1953. Review of the literature on dormancy in buds of woody plants. Meded. Landbouwhogesch. Wageningen, **53**: 1–24.

Eagles, C.F., and Wareing, P.F. 1964. The role of growth substances in the regulation of bud dormancy. Physiol. Plant. **34**: 201–203.

Evans, L.T., King, R.W., Mander, L.N., Pearce, D.W., and Pharis, R.P. 1993. Method of treating plants and plant tissues. Int. Patent Appl. PCT/Au92/00426, filed August 1992. Intl. Publ. No. WO 93/03616 (4 March 1993).

Evans, L.T., King, R.W., Mander, L.N., and Pharis, R.P. 1994*a*. The relative significance for stem elongation and flowering in *Lolium temulentum* of 3β-hydroxylation of gibberellins. Planta, **192**: 130–136.

Evans, L.T., King, R.W., Mander, L.N., Pharis, R.P., and Duncan, K.A. 1994*b*. The differential effects of C-16,17 dihydro gibberellins and related compounds on stem elongation and flowering in *Lolium temulentum*. Planta, **193**: 107–114.

Foster, K.R., Lee, I.J., Pharis, R.P., and Morgan, P.W. 1997. Effects of ring D-modified gibberellins on gibberellin levels and development in selected *Sorghum bicolor* maturity genotype. J. Plant Growth Regul. **16**: 79–87.

Fuchigami, L.H., Hotze, M., and Weiser, C.J. 1977. The relationship of vegetative maturity to rest development and spring budbreak. J. Am. Soc. Hortic. Sci. **102**: 450–452.

Goto, N., and Pharis, R.P. 1999. Investigations on the role of gibberellins in the development of floral organs of the gibberellin-deficient mutant, ga1–1, of *Arabidopsis thaliana*. Can. J. Bot. **77**: 944–954.

Hatch, A.H., and Walker, D.R. 1969. Rest intensity of dormant peach and apricot leaf buds as influenced by temperature, cold hardiness and respiration. J. Am. Soc. Hortic. Sci. **94**: 304–307.

Hemberg, T. 1949. Growth inhibiting substances in terminal buds of *Fraxinus*. Physiol. Plant. **2**: 37–44.

Hendershott, C.H., and Walker, D.R. 1959. Identification of a growth inhibitor from extracts of dormant peach flower buds. Science (Washington, D.C.), **130**: 798–799.

Hoad, G.V., MacMillan, J., Smith, V.A., Sponsel, V.M., and Taylor, D.A. 1982. Gibberellin 2β-hydroxylase and biological activity of 2β-alkyl gibberellins. *In* Plant growth substances. *Edited by* P.F. Wareing. Academic Press, London and New York. pp. 91–100.

Howard, W.L. 1951. An experimental study of the rest period in plants. Mo. Agric. Exp. Stn. Res. Bull. **21**: 1–74.

Lockhart, J.A., and Bonner, J. 1957. Effect of gibberellic acid on the photoperiodically controlled growth of woody plants. Plant Physiol. **32**: 492–499.

Looney, N.E., and Pharis, R.P. 1985. Gibberellins and reproductive development of tree fruits and grapes. Acta Hortic. (Wageningen), **179**: 59–72.

Luna, V. 1993. Aspectos morfológicos, fenológicos hormonales y metabólicos de la dormición en yemas florales de duraznero (*Prunus persica* L.). Doctoral thesis, Universidad Nacional de Río Cuarto, Río Cuarto, Argentina.

Luna, V., Lorenzo, E., Reinoso, H., Tordable, M.C., Abdala, G., Pharis, R.P., and Bottini, R. 1990. Dormancy in peach (*Prunus persica* L.) flower buds. I. Floral morphogenesis and endogenous gibberellins at the end of the dormancy period. Plant Physiol. **93**: 20–25.

Luna, V., Reinoso, H., Lorenzo, E., Bottini, R., and Abdala, G. 1991. Dormancy in peach (*Prunus persica* L.) flower buds. II. Comparative morphology and phenology in floral and vegetative buds, and the effect of chilling and gibberellin A_3. Trees (Berl.), **5**: 244–246.

Luna, V., Soriano, M.D., Bottini, R., Sheng, C.X., and Pharis, R.P. 1993. Dormancy in peach (*Prunus persica* L.) flower buds. III. Levels of endogenous gibberellins, abscisic acid, indole-3-acetic acid, and naringenin during dormancy of peach flower buds. Acta Hortic. (Wageningen), **329**: 264–267.

Mander, L.N., Sherburn, M., Camp, D., Evans, L.T., King, R.W., and Pharis R.P. 1998. Further studies on D-ring modified gibberellins and their influence on flowering and stem elongation in *Lolium temulentum*. Phytochemistry, **49**: 2195–2206.

Martin, G.C. 1991. Bud dormancy in deciduous fruit trees. *In* Plant physiology: a treatise. Vol. X. Growth and development. *Edited by* F.C. Steward and R.G.S. Bidwell. Academic Press, New York. pp. 183–225.

Nitsch, J.P. 1957. Photoperiodism in woody plants. Proc. Am. Soc. Hortic. Sci. **70**: 526–544.

Ohkuma, K., Addicott, F.T., Smith, O.E., and Thiesen, C.W. 1965. The structure of abscisin II. Tetrahedron Lett. **29**: 2529–2535.

Paiva, E., and Robitaille, H.A. 1978. Breaking bud rest on detached apple shoots: effects of wounding and ethylene. J. Am. Soc. Hortic. Sci. **103**: 101–104.

Perry, T.O. 1971. Dormancy of trees in winter. Science (Washington, D.C.), **171**: 29–36.

Phillips, I.D.J. 1962. Some interactions of gibberellic acid with naringenin 5,7,4′-trihydroxiflavanone in the control of dormancy and growth. J. Exp. Bot. **13**: 213–226.

Powell, L.E. 1987. Hormonal aspects of bud and seedling dormancy in temperate-zone woody plants. HortScience, **22**: 845–850.

Rademacher, W., Temple-Smith, K.E., Griggs, D.L., and Hedden, P. 1992. The mode of action of acylcyclohexanediones, a new type of growth retardant. *In* Progress in plant growth regulation. *Edited by* C.M. Karssen, L.C. van Loon, and D. Vreugdenhil. Kluwer Academic Publishers, Dordrecht, Netherlands. pp. 571–577.

Reinoso, H., Luna, V., Pharis, R.P., and Bottini, R. 2002. Dormancy in peach (*Prunus persica*) flower buds. V. Anatomy of bud development in relation to phenological stage. Can. J. Bot. **80**: 656–663.

Samish, R.M. 1954. Dormancy in woody plants. Annu. Rev. Plant Physiol. **5**: 183–204.

Saure, M.C. 1985. Dormancy release in the deciduous fruit trees. Hortic. Rev. **7**: 239–300.

Smith, H., and Kefford, N.P. 1964. The chemical regulation of the dormancy phases of bud development. Am. J. Bot. **51**: 1002.

Takagi, M., Pearce, D.W., Janzen, L.M., and Pharis, R.P. 1994. Effect of C-16,17 exo-dihydro gibberellin A_5 on gibberellin A_{20} metabolism in seedlings of dwarf rice (*Oryza sativa* L. cv. Tanginbozu). Plant Growth Regul. **15**: 207–213.

Walker, D.R., and Donoho, C.W. 1959. Further studies on the effect of gibberellic acid on breaking the rest period of young peach and apple trees. Proc. Am. Soc. Hortic. Sci. **74**: 87–92.

Walser, R.H., Walker, D.R., and Seeley, S.D. 1981. Effect of temperature, fall defoliation, and gibberellic acid on the rest period of peach leaf buds. J. Am. Soc. Hortic. Sci. **106**: 91–94.

Wareing, P.F., and Saunders, P. 1971. Hormones and dormancy. Annu. Rev. Plant Physiol. **22**: 261–298.

Xu, Y.-L., Li, L., Gage, D.A., and Zeevaart, J.A.D. 1999. Feedback regulation of GA_5 expression and metabolic engineering of gibberellin levels in *Arabidopsis*. Plant Cell, **11**: 927–935.

Zhou, R., and Pharis, R.P. 1998. Ring D-modified GA_5 and its derivatives competitively inhibit recombinant *Arabidopsis* GA 3β-hydroxylase. Poster 292, 16th International Plant Growth Substances Association Conference, Tokyo, Japan, August 13–17, 1998. International Plant Growth Substances Association, Agricultural Chemistry Department, University of Tokyo, Tokyo, Japan. Book of abstracts. p. 157.

Zimmermann, R.H., Hackett, W.P., and Pharis, R.P. 1985. Hormonal aspects of phase change and precocious flowering. Encycl. Plant Physiol. New Ser. **11**: 79–105.